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Measurement of Nitroaromatic Explosives by Micellar Electrokinetic Chromatography in Waters Collected Along a Tropical Estuary

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14. ABSTRACT

Novel methods for detecting nitroaromatic explosives use groundwater, seawater, or a mixture of seawater and MilliQ water. Micellar electrokinetic chromatography (MEKC) was used to detect nitroaromatic compounds added to samples collected from a tropical estuary (Kahana Bay, Oahu, HI). This is the first report measuring low levels of energetics added into natural water samples collected along an estuarine salinity transect (2, 5, 8, 15, 19, 27, 32, and 35 PSU). In addition to salinity differences among samples, dissolved organic carbon concentration ranged from 0.8 to 2.4 mg C L^{-1}. No attenuation over the 0 h incubation time was seen for 2,4,6-Trinitrotoluene, RDX, HMX, 1,3,5-Trinitrobenzene, 1,3-Dinitrobenzene, Dinitrotoluene, and Amino-dinitrotoluene. There were more significant losses (50% to 60% d^{-1}) for 2-, 3- and 4-nitrotoluene, and nitrobenzene. Little effect of salinity or dissolved organic carbon concentration was seen among these natural water samples. Because all samples were filtered to remove the natural bacterial assemblage, losses of 2-, 3- and 4-nitrotoluene and nitrobenzene were attributed to abiotic processes. Lack of attenuation of 2,4,6-Trinitrotoluene, 1,3,5-Trinitrobenzene, and 1,3-Dinitrobenzene contrasts with other reports for unfiltered, natural river water samples suggesting that these compounds are metabolized by natural bacterial assemblages within the stored sample.

15. SUBJECT TERMS

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MEKC	DOC	Estuarine water
Bacteria	Salinity	Seawater

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1. Introduction

US DOD has hundreds of sites involving estimated tens of millions of acres containing unexploded ordnance and energetic compounds that are either in coastal waters or can impact the watershed feeding estuarine ecosystems [1, 2]. Nitrogenous energetics leaking from unexploded, or partially-detonated, ordnance can impact local surface and groundwater and migrate to nearby rivers and estuaries resulting in episodic or chronic low level exposure to biota. Many of the parent compounds (TNT, RDX, HMX) and partial degradation products are known human and ecological health hazards (see review [3]).

Characterization of most DOD sites is just getting underway and the standard method for measuring energetics [4] is labor intensive and can require large amount of sample (i.e. 1 L). Thus, there have been many efforts to develop alternative detection methods for use with natural water samples from coastal environments including immunological [5, 6], quartz crystal microbalance [7], fiber microextraction [8-10], electrochemical diamond [11], polymer-oligopeptide composite [12], dicyclohexylamine-based spectrophotometric [13], fluorescence-quenching transduction [14] and numerous capillary electrophoretic methods [15]. MEKC separation and detection of energetics has recently been shown to be useful in direct sampling from seawater [16].

Many recent methods have been validated for environmental applications using laboratory buffer, groundwater or artificial seawater as the sampling medium (e.g. [6]). Estuarine waters that have salinity that is intermediate between river (0 PSU) and seawater (35 PSU) are often approximated by diluting full strength seawater with MilliQ water (e.g. [17]). However, natural estuarine water is a mix of river water (or groundwater from intrusion) with seawater. Estuarine samples have organic or inorganic components that are either an average of end members (conservative mixing, e.g. sodium chloride) or elevated or depleted (nonconservative; e.g. dissolved organic carbon (DOC)). Typically DOC is much higher in river water relative to seawater but can be changed (e.g. chemical composition, concentration) by natural microbial assemblages as water mixes along the estuary [18]. Recent work has proposed that some

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biogeochemical characteristics of natural river water may affect attenuation rates of nitroaromatics during sample storage [19]. Their variable presence may also act as interferants specific to a given energetic detection analysis.

This study determined whether coastal water samples of various ionic strength and DOC concentration would systematically affect detection of nitroaromatics by MEKC. Effects of other laboratory procedures on nitroaromatic detection were also examined including storage temperature (RT verses 4^{o}C) and in light verses dark. Such findings may constrain MEKC use with environmental samples.

2. Materials and Methods

2.1 Study site and sampling

Kahana Bay is a tropical ecosystem with a defined and persistent salinity gradient from the fresh/brackish Kahana Stream to the Pacific ocean over a relatively short transect (a few hundred meters) [20]. Dramatic differences in stream flow (as monitored by a USGS river station) affect freshwater end member salinity and location of mixing areas. During samplings on 20 July 2010 (5, 8, 15, 19, 27, 32 PSU) and 1 August 2011 (2, 35 PSU), river outflow (5 PSU) mixed with estuarine water over a shallow (0.5-1 m deep) shoal extending from the Kahana Stream mouth into the bay (Figure 1). Surface water was collected (1 L polycarbonate bottles) by hand, while wading from shore. They were filtered (0.22 μm nom. pore dia.) within 3 h, placed on ice for overnight shipping to the chromatography lab and then stored (4^{o}C) in the dark. Salinity was measured using a hand-held refractometer with sampling locations based on a previous study [21].

2.2 Standard solutions and analyses

Individual energetic standards including HMX, RDX, 1,3,5-Trinitrobenzene, 1,3-Dinitrobenzene, Tetryl, Nitrobenzene, 2,4,6-Trinitrotoluene, 4-Amino-2,6-Dinitrotoluene, 2- Amino-4,6-Dinitrotoluene, 2,4-Dinitrotoluene, 2,6- Dinitrotoluene, 2-Nitrotoluene, 3- Nitrotoluene, and 4- Nitrotoluene (Cerilliant,

Round Rock, TX) were diluted from stock concentration (1000 μg mL^{-1} in acetonitrile) in 10 mL of sample that was re-filtered (0.45 μm nom. pore dia.) in the laboratory into 20 mL borosilicate scintillation vials (final analyte concentration: 5 μg mL^{-1}). Of the 10 mL, 1.6 mL was used within 4 h for MEKC analyses while the remainder was either stored in ambient lab light (RT) or in the dark (4°C). Samples were analyzed by a modification of the MEKC method of Giordano et al. [16], where electrokinetic injection was replaced with a hydrodynamic injection resulting in a sample plug length of 1 cm. Given the large difference between separation media conductivity and sample matrix conductivity, relative standard deviation for repeated injection of the same sample for stable analytes was 10%. DOC concentration was determined by wet chemical oxidation [22].

3. Results and Discussion

Nitroaromatic compounds were quantified by an MEKC method that has been shown to be useful with full strength seawater [16]. These standards were added to filtered water samples that were collected along a tropical estuarine salinity gradient to determine effect of natural water chemistry on nitroaromatic quantification. Vials containing these standards were sampled six times over ca. 40 h (e.g. 35 PSU electropherogram, Figure 2; elution order, Table 1). Two pairs of analytes are not resolved using these separation conditions; 2-Nitrotoluene and 3-Nitrotoluene co-migrate, as do 2-Am-4,6-DNT and 4-Am-2,6-DNT. There did not appear to be any effect of salinity, DOC concentration, or other unmeasured geochemical feature on peak area over time for any energetic standard, save for Tetryl. Reproducibility across the salinity gradient of 30% for Tetryl peak area far exceeds accepted instrumentation variability (~10%) and may be due to its poor aqueous solubility. This variability occurred throughout the time course, making it difficult to discern any trends associated with Tetryl as a function of either time or salinity. When sampled six times over 40 h, most nitroaromatics showed no effect of salinity and no attenuation relative to their starting concentration: 1,3,5-Trinitrobenzene, HMX, 2,4,6-Trinitrotoluene, RDX, 1,3-Dinitrobenzene, 2,4-Dinitrotoluene, 2,6-Dinitrotoluene and the Amino-Dinitrotoluenes (Table 1). Results for HMX, RDX and the Amino-Dinitrotoluenes were similar to those reported by Douglas et

al.[19] using glacial melt river water. However, they reported relative attenuation rates of 1,3,5-Trinitrobenzene > 2,4,6-Trinitrotolene > 1,3-Dinitrobenzene whereas those compounds appeared stable under conditions of our study even when normalized for incubation time.

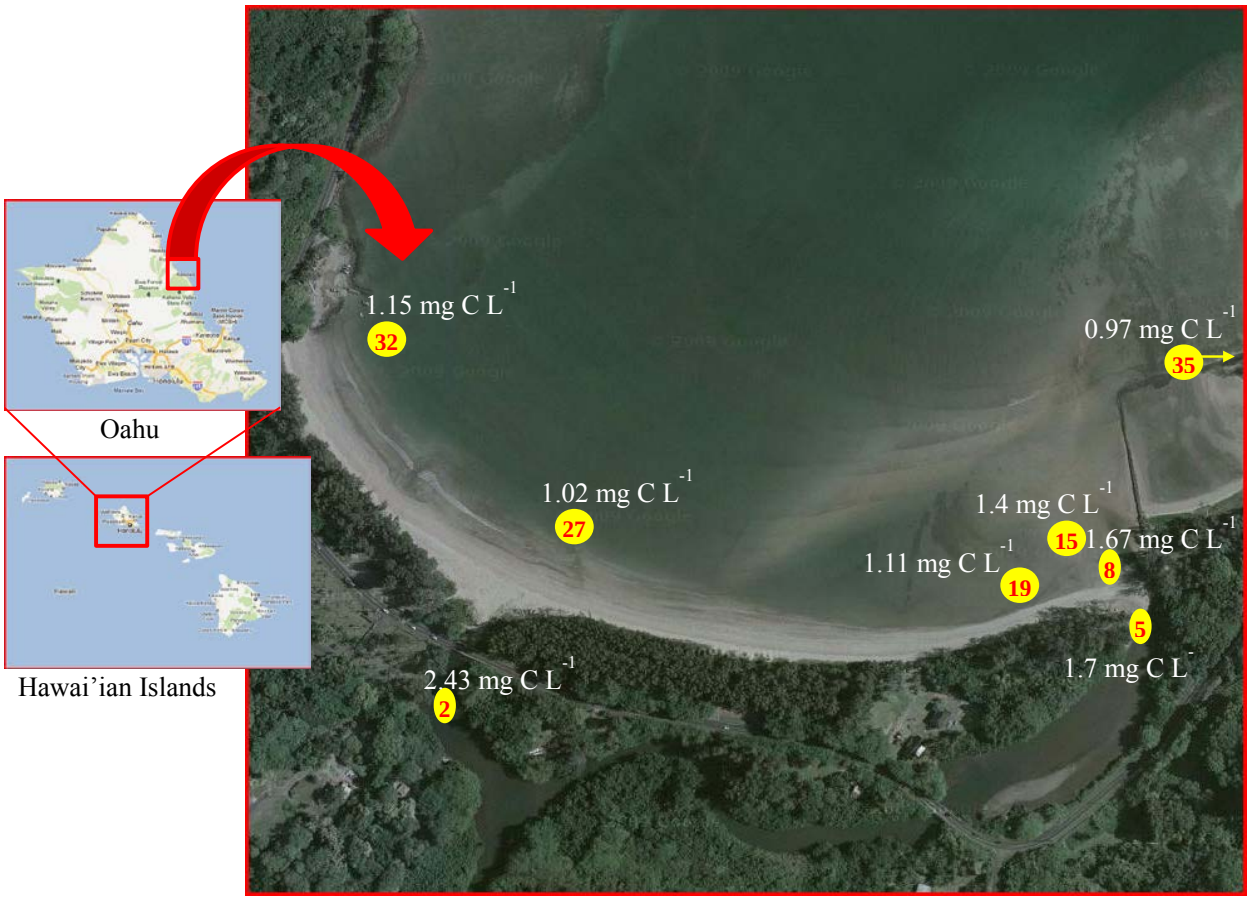

Figure 1. Sampling stations for a salinity transect from the Kahana Stream (2, 5) and Bay (8, 15, 19, 27, 32), Oahu, HI, USA and Pacific Ocean (35). Station designations refer to their salinity (PSU) along with DOC concentration (mg C L^{-1}; Google maps).

The primary difference among natural samples between the two studies was presence of the natural bacterial assemblage and particulate matter (organic and inorganic) in Douglas et al. [19] as their samples were unfiltered. Particulate organic carbon could play a role in abiotic attenuation of nitroaromatics but it was similar among two waterways, Bear Creek (3.2 mg L^{-1}) and Jarvis Creek (2.5 mg L^{-1}), that had large differences in attenuation [19]. Difference in particulate inorganic matter also did not correlate well with observed attenuation of the four nitroaromatics among the water body samples with the highest rate, Bear Creek, and that with the highest particulate inorganic matter, Chena River [19]. These results suggest that other factors were more important in attenuating nitroaromatics.

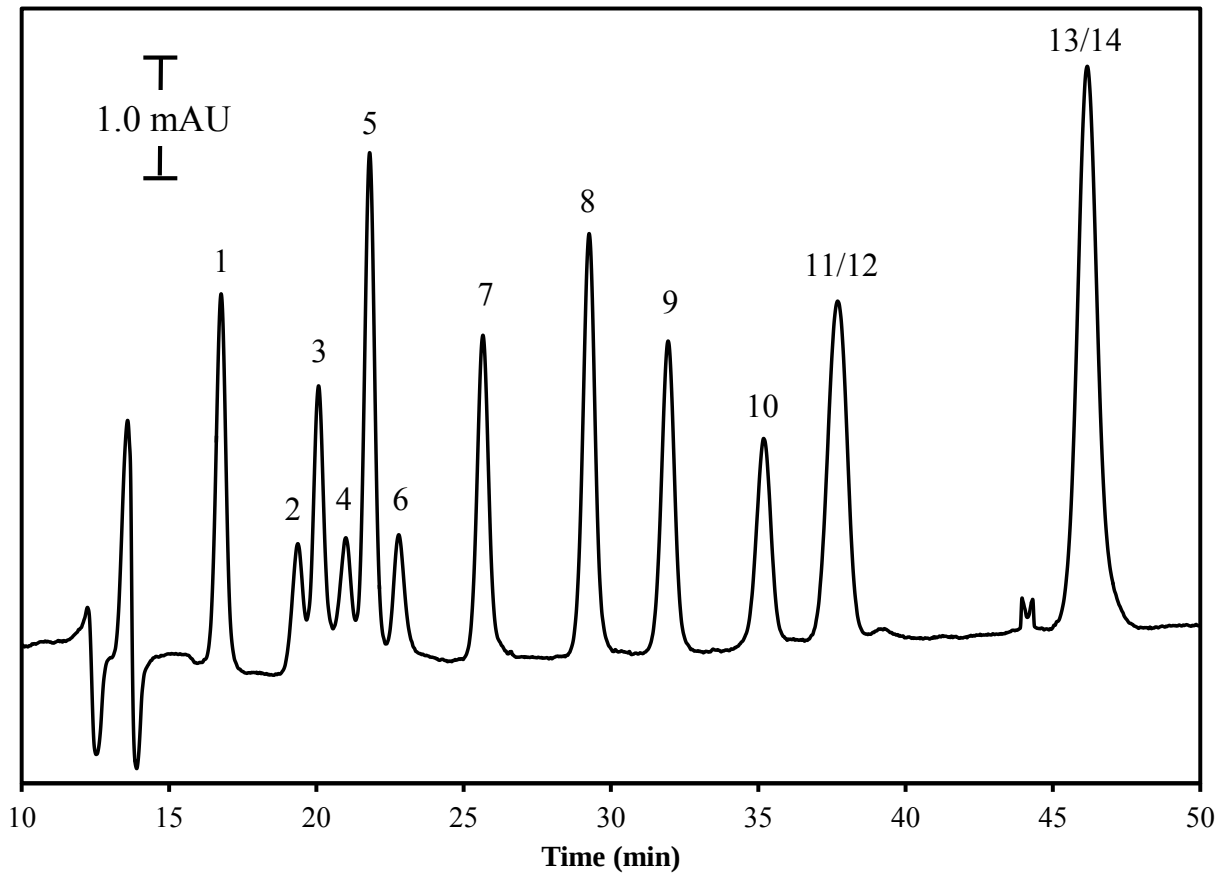

Figure 2. Representative electropherogram for a mixture of 14 nitroaromatics, nitramines and their degradation products. Sample concentration was 5 μg mL^{-1} for all analytes. See Table 1 for compound listing.

DOC concentration was much higher among glacial watersheds with the highest attenuation rates for those nitroaromatics that changed concentration over their 85 d incubation [19]. DOC presence alone may

not explain the difference among our studies as the range for our tropical estuary study, 0.97-2.43 mg L^{-1} (Figure 1), was within that for the glacial watershed [23], <1.5-11.3 mg L^{-1}. The most likely candidate is presence of a natural bacterial assemblage in unfiltered glacial watershed samples. Bacterial growth rate was not measured in Douglas et al. [19] samples but DOC supports bacterial production in glacial watersheds, as well as, estuarine waters [24]. In a related study of Kahana Bay, heterotrophic bacterial production positively correlated ($R^2 = 0.79$) with DOC concentration [24]. 2,4,6-Trinitrotoluene loss in Douglas et al. [19] (ca. 0.5 µg L^{-1} d^{-1}) is similar to that reported for 2,4,6-Trinitrotoluene mineralization by natural assemblages at Kahana Bay (0.1-0.2 µg L^{-1} d^{-1}) [24]. This is evidence that natural assemblages from glacial watersheds can metabolize nitroaromatics like 1,3,5-Trinitrobenzene, 2,4,6-Trinitrotoluene, and 1,3-Dinitrobenzene.

Table 1. Effect of salinity (PSU) on peak area four hours after addition of nitroaromatic compounds to the sample matrix (salinity gradient). Compounds are listed in order of elution[a] by MEKC[b].

		Peak Areas								**Normalized for Total Peak Area**							
		PSU								**PSU**							
Peak	**Compound**	**2.6**	**5**	**8**	**15**	**19**	**27**	**32**	**35**	**2.6**	**5**	**8**	**15**	**19**	**27**	**32**	**35**
1	1,3,5-TNB	1150	1260	1160	1250	1180	1340	1330	1480	0.096	0.105	0.103	0.109	0.112	0.123	0.121	0.123
2	HMX	660	580	540	530	530	510	510	580	0.055	0.048	0.048	0.046	0.050	0.047	0.047	0.048
3	2,4,6-TNT	920	990	870	940	850	870	910	1000	0.077	0.082	0.078	0.082	0.081	0.080	0.083	0.083
4	RDX	600	600	520	530	520	500	530	530	0.050	0.050	0.046	0.046	0.049	0.046	0.048	0.044
5	1,3-DNB	1830	1850	1730	1690	1570	1600	1620	1750	0.154	0.154	0.154	0.147	0.149	0.147	0.148	0.145
6	Tetryl	470	280	210	250	260	210	300	450	0.039	0.023	0.019	0.022	0.025	0.019	0.027	0.037
7	NB	850	940	900	920	790	800	810	860	0.071	0.078	0.080	0.080	0.075	0.074	0.074	0.071
8	2,4-DNT	1260	1280	1240	1210	1130	1180	1150	1250	0.106	0.107	0.111	0.106	0.107	0.109	0.105	0.104
9	2,6-DNT	880	910	880	850	790	840	820	910	0.074	0.076	0.079	0.074	0.075	0.077	0.075	0.076
10	4-NT	500	530	520	520	470	480	470	500	0.042	0.044	0.046	0.045	0.045	0.044	0.043	0.042
11/12	3-NT/2-NT	940	980	960	1010	850	860	890	930	0.079	0.082	0.086	0.088	0.081	0.079	0.081	0.077
13/14	2-Am-4,6-DNT/ 4-Am-2,6-DNT	1860	1810	1680	1760	1610	1680	1620	1800	0.156	0.151	0.150	0.154	0.153	0.155	0.148	0.150

[a] 1,3,5-Trinitrobenzene (1,3,5-TNB), HMX, 2,4,6-Trinitrotoluene (2,4,6-TNT), RDX , 1,3-Dinitrobenzene (1,3-DNB), Tetryl, Nitrobenzene (NB), 2,4-Dinitrotoluene (2,4-DNT), 2,6-Dinitrotoluene (2,6-DNT), 4- Nitrotoluene (4-NT), 2,3-NTs – co-migrating, and Amino-Dinitrotoluenes (Am-DNTs) - comigrating.

[b] Values for individual compound peaks were also normalized against total peak area.

Lack of 2,4,6-Trinitrotoluene attenuation in our study seems to contrast with that of Harrison and Vine [25] using sterilized sediment, however, those workers used sodium azide which is known to be a poor inhibitor of lignolytic microorganisms capable of metabolizing aromatic organic contaminants [26]. Other compounds showed little effect of salinity but did attenuate to various degrees after 40 h, including 70-90% decrease for Nitrobenzene, 4-Nitrotoluene, and the 2,3-Nitrotoluenes (Figures 3,4,5). Given that such decreases occurred in filtered water samples, these nitroaromatics were most likely attenuated by abiotic processes (e.g. volatilization, chemical transformation, salting out). It should also be noted, that there was no difference (in excess of 10% instrument variance) in final concentration for all analytes after 40 h of incubation when either stored at 4^{o}C in the dark or at RT in a clear glass vial on the laboratory bench top (under room lights) suggesting that photodegradation was not an issue under these storage and analyses conditions.

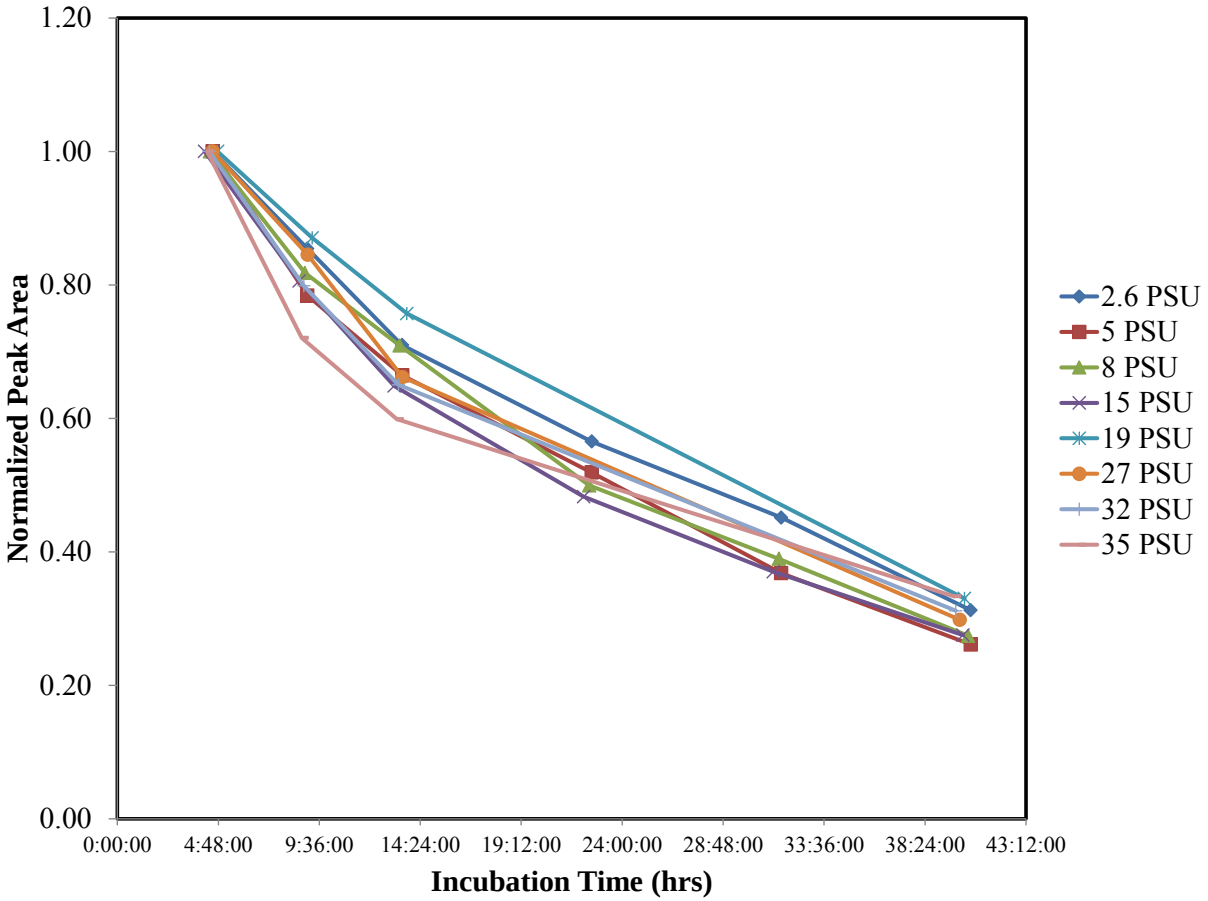

Figure 3. Peak area over time normalized to peak area determined 4 hours after initial spiking of sample matrix with analytes for Nitrobenzene.

4. Concluding remarks

Salinity and DOC differences along a coastal estuary from a freshwater river to Pacific Ocean seawater had no measurable effect on energetic detection by MEKC. Attenuation of 2,4,6-Trinitrotoluene, RDX, HMX, 1,3,5-Trinitrobenzene, 1,3-Dinitrobenzene, 2,4-Dinitrotoluene, 2,6-Dinitrotoluene and the Amino-Dinitrotoluenes did not occur in the absence of natural microbial assemblages over the 40 h incubation period. 4-Nitrotoluene, the 2-,3-Nitrotoluenes, and Nitrobenzene did show measure of attenuation suggesting that removal was due to abiotic processes. MEKC is a useful tool for detecting most energetic compounds from coastal estuarine environments.

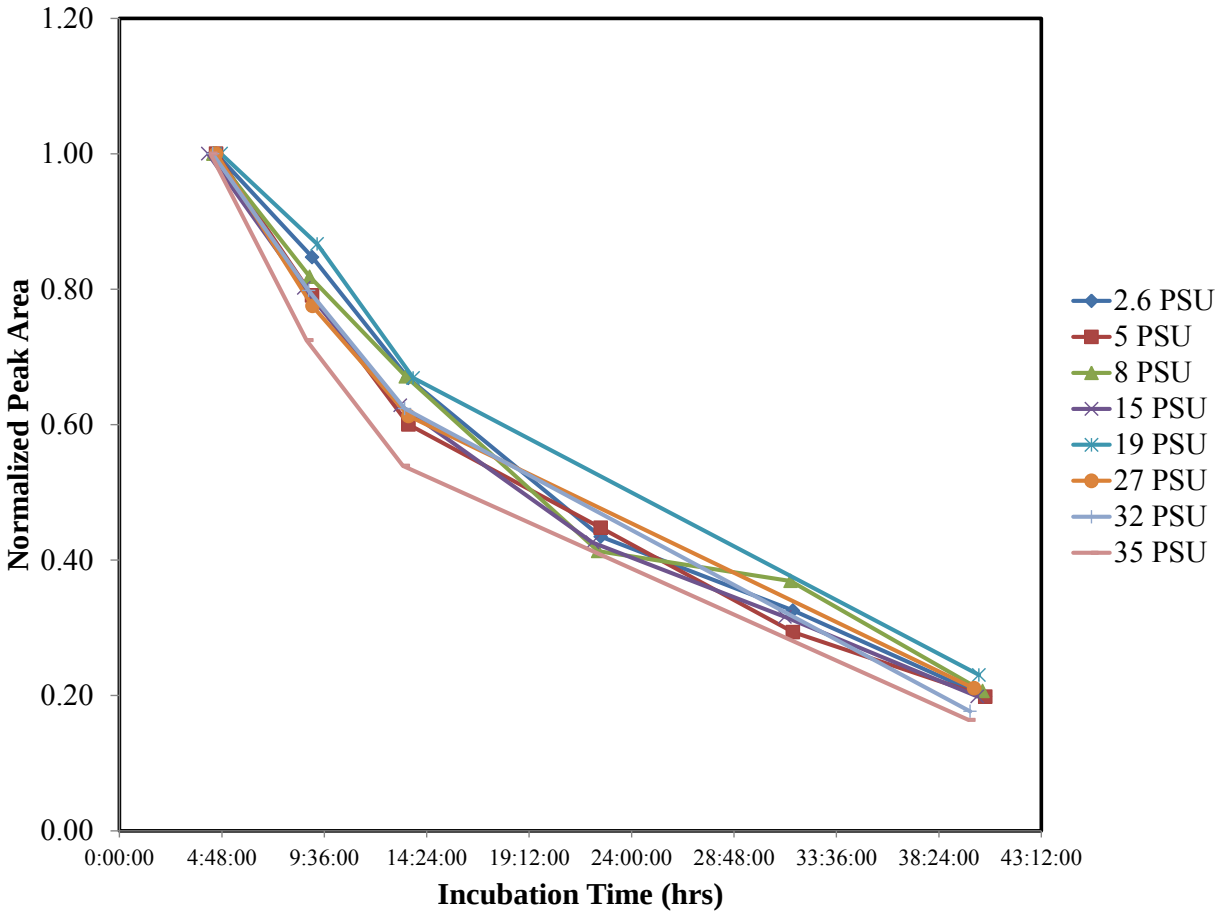

Figure 4. Peak area over time normalized to peak area determined 4 hours after initial spiking of sample matrix with analytes for 4-Nitrotoluene.

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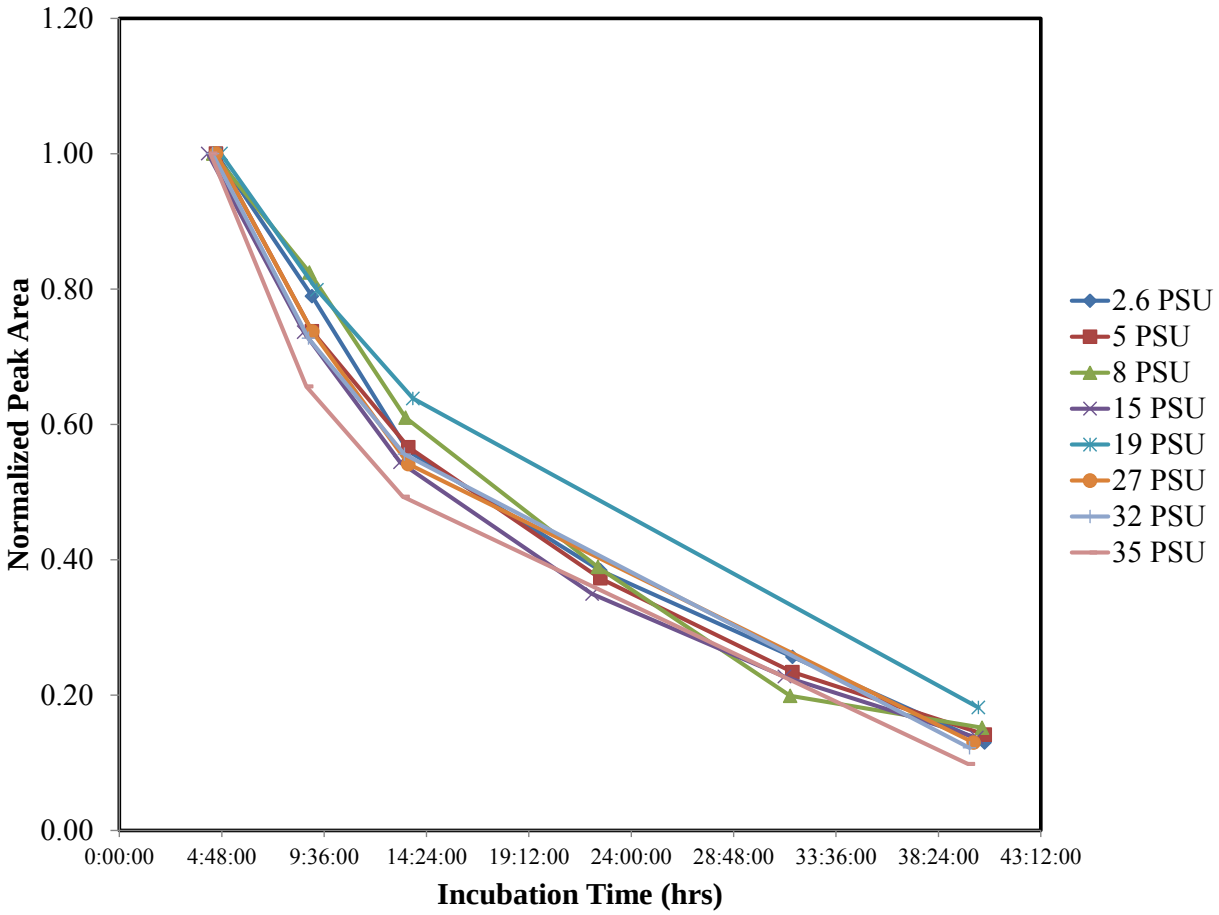

Figure 5. Peak area over time normalized to peak area determined 4 hours after initial spiking of sample matrix with analytes for peak associated with 2-Nitrotoluene and 3-Nitrotoluene.

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References

[1] Government Accounting Office, *Military Munitions: DOD Needs to Develop a Comprehensive Approach for Cleaning up Contaminated Sites. Report to Honorable John D. Dingell, Ranking Minority Member, Committee on Energy and Commerce, House of Representatives,* GAO-04-147, 2003. http://www.gao.gov/new.items/d04601.pdf.

[2] Strategic Environmental Research and Development Program, *Final Report: SERDP and ESTCP Workshop on Technology Needs for the Characterization, Management, and Remediation of Military Munitions in Underwater Environments,* Arlington, VA, USA, 2007. http://www.serdp.org/content/download/8242/101248/file/MM-UW-Workshop-Report-2007.pdf.

[3] Roldan, M. D., Perez-Reinado, E., Castillo, F., Moreno-Vivian, C., *FEMS Microbiol. Rev.* 2008, 32, 474-500.

[4] EPA Method 8330, Environmental Protection Agency, Government of the United States. www.epa.gov/osw/hazard/testmethods/sw846/pdfs/8330a.pdf.

[5] Charles, P. T., Rangasammy, J. G., Anderson, G. P., Romanoski, T. C., Kusterbeck, A. W., *Anal. Chim. Acta* 2004, 525 (2), 199-204.

[6] Bromage, E. S., Vadas, G. G., Harvey, E., Unger, M. A., Kaattari, S. L., *Environ. Sci. Technol.* 2007, 41, 7067-7072.

[7] Harbeck, M., Erbahar, D. D., Gurol, I., Musluaoglu, E., *Instrumentation and Measurement Technology Conference (I2MTC),* IEEE, 3-6 MAY 2010, 111-115. http://dx.doi.org/10.1109/IMTC.2010.5488240.

[8] Psillakis, E., Mantzavinos, D., Kalogerakis, N., *Anal. Chim. Acta* 2004, 501(1), 3-10.

[9] Monteil-Rivera, F., Beaulieu, C., Hawari, J., *J. Chromatogr. A* 2005, 1066 (1-2), 177-187.

[10] Perr, J. M., Furton, K. G., Almirall, J. R., *J. Sep. Sci.* 2005, 28 (2), 177-183.

[11] de Sanoit, J., Vanhove, E., Mailley, P., Bergonzo, P., *Electrochim. Acta* 2009, 54, 5688-5693.

[12] Cerruti, M., Jaworski, J., Raorane, D., Zueger, C., Varadarajan, J., Carraro, C., Lee, S., Maboudian, R., Majumdar, A., *Anal. Chem.* 2009, 81, 4192-4199.

[13] Erçag, E., Üzer, A., Apak, R., *Talanta* 2009, 78, 772-780.

[14] Harper, R. J., Dock, M. L., *Proc. SPOI* 2007, 6540, 65400V. http://dx.doi.org/10.1117/12.719742.

[15] Du, Y., Wang, E., *J. Sep. Sci.* 2007, 30, 875-890.

[16] Giordano, B. C., Burgi, D. S., Collins, G. E., *J. Chromatogr. A* 2010, 1217, 4487-4493.

[17] Giordano, B. C., Copper, C. L., Collins, G. E., *Electrophoresis* 2006, 27, 778-786.

[18] Bianchi, T. S., *Biogeochemistry of Estuaries,* Oxford University Press, New York, 2007.

[19] Douglas, T. A., Johnson, L., Walsh, M., Collins, C., *Chemosphere* 2009, 76, 1-8.

[20] Maciolek, J. A., Timbol, A. S., *Bull. Mar. Sci.* 1981, 31, 712-722.

[21] Montgomery, M. T., Coffin, R. B., Boyd, T. J., Smith, J. P., Plummer, R. E., Walker, S. E., Osburn, C. L., *Environ. Poll.* 2011, 159, 3673-3680.

[22] Osburn, C. L., St-Jean, G., *Limnol. Oceanogr. Meth.* 2007, 5, 296-308.

[23] Hood, E., Fellman, J., Spencer, R. G. M., Hernes, P. J., Edwards, R., D'Amore, D., Scott, D., *Nature* 2009, 462, 1044-1047.

[24] Montgomery, M. T., Coffin, R. B., Boyd, T. J., Osburn, C. L., Naval Research Laboratory, Technical Memorandum Report, 2012, NRL/MR/6110—12-9390. https://torpedo.nrl.navy.mil/tu/ps/pdf/pdf_loader?dsn=14019196

[25] Harrison, I., Vane, C., *Water Sci. Technol.* 2010, 61 (10), 2531-2538.

[26] Verdin, A., Lounes-Hadj Sahraoui, A., Durand, R., *Int. J. Biodeter. Biodegrad.* 2004, 53, 65-70.